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A / Box Sequence

Docket No. 95-97

PATENT APPLICATION TRANSMITTAL LETTER

To the Commissioner of Patents and Trademarks:

Transmitted herewith for filing under 35 U.S.C. 111 and 37 C.F.R. §1.53 is the patent application of:
Guang-Jer Wu

entitled: **DIAGNOSTIC FOR METASTATIC PROSTATE CANCER.**

CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as Express Mail in an envelope addressed to: The Assistant Commissioner for Patents Washington, D.C., 20231	
Date	September 1, 2000
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Enclosed are:

- ☒ 26 pages of written description, claims and abstract
- ☒ 6 sheets of drawings
- ☒ 17 pages of Sequence Listing as paper copy
- ☐ an assignment of the invention to _____
- ☒ an executed declaration of the inventors and power of attorney
- ☐ a certified copy of a _____ application
- ☐ associate power of attorney
- ☒ a verified statement to establish small entity status under 37 CFR §1.9 and §1.27 (executed)
- ☐ information disclosure statement
- ☐ preliminary amendment
- ☒ Sequence Listing diskette, Statement under 37 C.F.R. 1.821-1.824

1c714 U.S. PTO
09/653961
09/01/00

CLAIMS AS FILED

	Number Filed	Number Extra	Rate	Fee
BASIC FEE			\$690	\$690
TOTAL CLAIMS	19 -20 =		x \$18	0
INDEPENDENT CLAIMS	4 - 3 =		x \$78	\$78
MULTIPLE DEPENDENT CLAIM PRESENT			x \$260	0

* Number extra must be zero or larger

TOTAL \$768

If applicant has small entity status under 37 C.F.R. 1.9 and 1.27, then divide total fee by 2, and enter amount here.

SMALL ENTITY TOTAL

\$384

☐ No filing fee is enclosed at this time.

☒ A check in the amount of **\$384.00** to cover the filing fee is enclosed.

☐ The Commissioner is hereby authorized to charge and credit Deposit Account No. 07-1969 as described below. A duplicate copy of this sheet is enclosed.

☐ Charge the amount of \$_____ as filing fee.

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☐ Charge any additional filing fees required under 37 CFR 1.16.

☐ Charge any processing fees required under 37 CFR 1.17.

☐ Charge the issue fee set in 37 CFR 1.18 at the mailing of the Notice of

☐ Allowance, pursuant to 37 CFR 1.311(b).

☐ Other _____

☒ **Benefit of Prior U.S. Application(s) (35 USC 120)**

Applicant claims priority under 35 USC 120 to the following application(s):

PCT US99/04850 filed 2 March 1999 and designating the United States

Benefit of Prior U.S. Provisional Application(s) (35 USC 119(e))

Applicant claims priority under 35 USC 119(e) to the following application(s):

60/076,664 filed 3 March 1998

☐ **Benefit of Prior Foreign Application(s) (35 USC 119)**

Applicant claims priority under 35 USC 119 to the following application(s):



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Docket No.: 95-97

nnr:9/1/00

Applicant or Patentee: Guang-Jer Wu
Serial or Patent No.:
For: Diagnostic for Metastatic Prostate Cancer

Attorney's # : 95-97 US
Filed or Issued:

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. 1.9(f) and 1.27(d)) - NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: EMORY UNIVERSITY
ADDRESS OF ORGANIZATION: 2009 Ridgewood Drive
Atlanta, GA 30322

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION LOCATED IN ANY COUNTRY
☐ AN ORGANIZATION OF THE TYPE DESCRIBED IN SECTION 501(c)(3) OF THE INTERNAL REVENUE CODE OF 1954 (26 U.S.C. 501(c)(3)) AND EXEMPT FROM TAXATION UNDER SECTION 501(a) OF THE INTERNAL REVENUE CODE (26 U.S.C. 501(a))
☐ ANY NONPROFIT SCIENTIFIC OR EDUCATIONAL ORGANIZATION QUALIFIED UNDER A NONPROFIT ORGANIZATION STATUTE OF A STATE OF THIS COUNTRY (35 U.S.C. 201(i))
☐ ANY NONPROFIT ORGANIZATION LOCATED IN A FOREIGN COUNTRY WHICH WOULD QUALIFY AS A NONPROFIT ORGANIZATION UNDER THE ABOVE PARAGRAPHS (2) OR (3) IF IT WERE LOCATED IN THIS COUNTRY

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees under §41(a) and (b) of Title 35, United States Code, with regard to the invention entitled "Diagnostic for Metastatic Prostate Cancer"

by inventor(s) Guang-Jer Wu

described in ☒ the specification filed herewith
☐ application serial no. _____ filed _____
☐ patent no. _____ issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e). NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

NAME _____
ADDRESS _____

- ☐ INDIVIDUAL
☐ SMALL BUSINESS CONCERN
☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 C.F.R. 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Mary L. Severson
TITLE IN ORGANIZATION: Director, Office of Technology Transfer
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SIGNATURE

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DATE

Aug 24, 2000

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APPLICATION FOR LETTERS PATENT

Inventor: Guang-Jer Wu

DIAGNOSTIC FOR METASTATIC PROSTATE CANCER

CERTIFICATE OF MAILING

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DIAGNOSTIC FOR METASTATIC PROSTATE CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of International Application PCT US99/04850, filed
5 March 2, 1999 and designating the United States, which application claims priority from
United States Provisional Application No. 60/076,664 filed March 3, 1998.

ACKNOWLEDGMENT OF FEDERAL RESEARCH SUPPORT

Not applicable.

BACKGROUND OF THE INVENTION

10 Prostate cancer accounts for about 1 in 10 cancer cases in the United States, and it is
the most often diagnosed cancer in males [Henderson et al. (1991) *Science* 254, 1131-1138].
While in many affected patients, the tumors are slow-growing and nonmetastatic, in others
the malignant prostate tumors are aggressive and metastasize. When prostate cancer
metastasizes, the prognosis for the patient is poor, especially without treatment.

15 To date, the most frequently used test for prostate cancer is the serum level of prostate
specific antigen (PSA) and the radionuclide bone scan for detecting prostate cancer
metastases before definitive therapy is initiated. However, the elevated level of PSA in serum
is not predictive of the pathologic stage of the prostate cancer or the presence of metastatic
disease. PSA, a serine protease, is not exclusively expressed in the epithelial cells of
20 metastatic prostate cancer, but it is also expressed in normal epithelial cells, primary tumors
and benign prostate hyperplasia.

The altered expression of cell-adhesion molecules has been correlated with metastasis of many cancers. Low or no expression of E-cadherin, a cell-adhesion molecule, has been found in high-grade prostate carcinoma, and this indicates a poor prognosis for those prostate cancer patients. However, the absence of an antigen is not very useful as a diagnostic marker for cancer metastasis.

MUC18 is a glycoprotein of about 113 kDa which serves as a cell adhesion molecule on the surface of melanoma cells, and it has been correlated with the ability of melanomas to metastasize [See, e.g., Lehmann et al. (1989) *Proc. Nat. Acad. Sci. USA* 86, 9891-9895; Luca et al. (1993) *Melanoma Res.* 3, 35-41; Johnson et al. (1996) *Curr. Top. Microbiol. Immunol.* 213, 95-105; Xie et al. (1997) *Cancer Res.* 57, 2295-2303; Tang and Honn (1994-1995) *Invasion Metas.* 14, 109-122; Rummel et al. (1996) *Cancer Res.* 56, 2218-2223]. MUC18 is also known as MCAM and CD146. MUC18 carries a carbohydrate modification known as HNK-1 or CD57 [Shih et al. (1994) *Cancer Res.* 54, 2514-2520]. Besides being associated with melanoma cells' ability to metastasize, MUC18 is also associated with normal vascular tissue, and on the smooth muscle of venules, and it expresses sporadically on capillary epithelium [Johnson, J. (1994-1995) *Invasion Metas.* 14, 123-130].

There is a longfelt need in the art for an improved diagnostic test for metastatic prostate cancer so that appropriate therapy can be initiated as soon as possible and so that the number of false positive results can be minimized.

SUMMARY OF THE INVENTION

The present invention provides an improved diagnostic test for prostate cancer which has a relatively high potential for metastasis or which has metastasized. This allows the physician to choose appropriate surgical, chemotherapeutic or radiation treatment regimens. This improved assay is based on the correlation of high levels of expression of the MUC18 coding sequence as measured by MUC18 mRNA or MUC18 protein. This expression can be detected at the transcriptional level, where mRNA levels are monitored, or detection of the MUC18 gene product at the translation level can be determined, for example, through the use of an immunoassay for the MUC18 protein. The source of the material for these tests is prostate biopsy tumor tissue (e.g., from a needle biopsy) from a patient needing a

determination of the metastatic potential of a prostate tumor or from cells from a prostate tumor.

Relative levels of transcriptional expression (mRNA) of the MUC18 coding sequence can be determined by Northern hybridization analysis or by quantitative reverse transcription polymerase chain reaction (RT-PCR) in normal and neoplastic prostate tissue samples and in biopsy material.

Translational expression of MUC18 can be determined by any of a number of adaptations of an immunoassay using antibody specific for the MUC18 cell surface antigen. The relative level of MUC18 can be determined by standard immunoassays using a MUC18-specific antibody preparation and a detection system suitable for the assay. Immunoassays can include, but are not limited to, immunofluorescence assays, radioimmunoassays, enzyme-linked immunosorbent assays, and Western (immuno) blot assays. In the context of the present invention, relative amounts of the MUC18 protein are determined in tissue samples (e.g., biopsy material).

It is a further object of the present invention to provide an antibody which inhibits prostate cancer metastasis. In particular, antibody specific to MUC18 prevents metastasis of prostate cancer cells.

Additional objects include vectors directing the expression of an immunogenic fragment of human MUC18 and the corresponding recombinantly expressed protein. As specifically exemplified, an immunogenic fragment of human MUC18 is encoded by the PvuII to XhoI fragment within the sequence given in Tables 1A-1B.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the results for RT-PCR amplified human MUC18 cDNA (left panel) and cloning of the whole cDNA and its fragments into a GST-fusion protein expression vector (right panel). RT-PCR amplification of human MUC18 cDNA from the poly(A)+RNA isolated and purified from a human melanoma cell line, Sk-Mel-28. Left panel: Lane (a) shows the expected PCR product of 1957 bp (as indicated by an arrow head). Lane (m1) shows the 1 kb ladders and lane (m2) the 123-bp ladders as DNA molecular size markers. Right panel: The plasmid map of the cloned whole human MUC18 cDNA and three fragments in a GST-fusion expression system.

Fig. 2 illustrates Northern blot analysis of expression of human MUC18 in different prostate cancer cell lines. Poly(A)+RNA was isolated from human melanoma cells SK-MEL-28 (SK), human melanocyte (M), and human prostate cancer cells PC-3 (PC-3), DU145 (DU), TSU-PR-1 (TSUPR1), and LNCAP (LNCAP). The size of the human MUC18 mRNA is 3.3 kb. The amount of poly(A)+RNA (2.5 to 10 μ g) is indicated as a number on top of each lane.

Fig. 3 depicts recombinant human MUC18-middle fragment. The plasmid map is shown in Fig. 1. The left panel shows the PAGE result and right panel the Superdex column purification. The GST-human MUC18 middle fragment fusion protein is shown after IPTG induction (a & b, indicated by a triangle on the left of the left panel). The fusion protein was first purified through a glutathione-Sepharose affinity column and then cleaved with the HRV-3C protease (left panel, lanes c-e). The affinity-purified recombinant human MUC18-middle fragment was then further purified through a Superdex column (right panel) to remove high molecular weight contaminants (peaks I and II, fractions 3-13). The final recombinant human MUC18-middle fragment protein is about 22 kDa (peak III, fractions 14-20), as indicated by a triangle on the right in the left panel.

Fig. 4 shows pGEX-6P, commercially available from Pharmacia Biotech, Piscataway, NJ. The specific multiple cloning site (MCS) sequences for pGEX-6P-1 (SEQ ID NO:11, encoded amino acids, SEQ ID NO:12), pGEX-6P-2 (SEQ ID NO:13, encoded amino acids, SEQ ID NO:14) and pGEX-6P-3 (SEQ ID NO:15, encoded amino acids, SEQ ID NO:16) are provided.

Fig. 5 provides diagrammatic illustrations of the whole cDNA sequence of human MUC18, its N-terminal fragment, middle fragment and C-terminal fragment, as cloned into the pGEX-6P-1 vector are provided. These fragments as cloned result in the expression of the N-terminal, middle and C-terminal fragments of the MUC18 protein. See also Tables 1A-1B for the locations of the relevant restriction sites in the cDNA sequence for human MUC18 and SEQ ID NO:1 for sequence.

Fig. 6 illustrates the results of Western blot analysis of huMUC18 protein expression in four prostatic cancer cell lines. Cellular extracts of four prostatic cancer cell lines were prepared, and the proteins were size-separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The anti-huMUC18 antibodies generated by immunization of the recombinant huMUC18-middle portion protein in chicken were used for Western blot

analysis. SK stands for cellular lysate prepared from human SK-Mel-28 cells (a), Tsu-PR1 cells (b and c), DU145 for DU145 cells (d and e), PC-3 for PC-3 cells (f and g), and LNCaP for LNCaP.FGC cells (h and i). The number over each lane indicates the amount of protein (μ g) loaded in each well. The huMUC18 protein band is indicated with an open triangle.

5 The numbers on the right-most lane indicate the protein molecular weight (kDa) rainbow markers (RPN800, Amersham).

Fig. 7 shows the results of Western blot analysis of human MUC18 protein in normal prostatic gland, normal primary human prostatic epithelial cells, and tissues of a patient with malignant prostatic cancer. Cellular lysates were prepared from normal prostatic gland (b),
10 cultured normal primary prostatic epithelial cells (c), and prostatic cancerous tissues from a patient with malignant prostatic cancers (e-g). Cellular extracts prepared from human SK-Mel-28 cells (a) and from DU145 cells (d) were shown as the positive controls. 20 μ g protein of each lysate was loaded per well. The numbers on the right-most lane indicate the protein molecular weight (kDa) rainbow markers (RPN800, Amersham).

DETAILED DESCRIPTION OF THE INVENTION

Prostate cancer accounts for about 10% of all cancer cases in the United States. It is now the most frequently diagnosed cancer in American males [Rinker-Schaeffer et al. (1993) *Cancer and Metas. Rev.* 12, 3-10]. In some patients, prostate cancers metastasize rapidly, killing the patient within one year of the initial clinical presentation. In contrast, some other prostate cancer patients show a relatively slow growth of the malignant tumor without metastasis. The majority of the histologically localized prostate cancers remain subclinical and never require treatment. Prostate cancer, when truly localized, can be cured by radical prostatectomy. While in many affected patients, the tumors are slow-growing and nonmetastatic, in others prostate cancer takes a more aggressive course. Unfortunately,
20 metastatic prostate cancer is a fatal disease without treatment.

At present, the test for serum levels of prostate specific antigen (PSA) and the radionuclide bone scan are the only diagnostic tests available before therapy is initiated. However, an elevated level of PSA observed in serum is not predictive of the pathological state of prostate cancer, nor is it correlated with metastatic prostate cancer. This is, at least in
30 part, because PSA, a serine protease, is not exclusively expressed in the epithelial cells of

metastatic prostate cancer, but it is also expressed in normal epithelial cells, primary prostate cancerous tumors and benign prostatic hyperplasia [Wood et al. (1994) *Cancer* 74, 2533-2540]. To date, it has not been possible to predict whether histologically detected localized tumors are likely to progress to clinical cancer or when these localized tumors are likely to metastasize to other sites within the body. Thus, there is an urgent need for biochemical markers which serve to identify prostate cancers which have progressed to a stage which requires immediate surgical removal and/or additional chemotherapeutic or radiation therapeutic treatments, i.e., those cancers which are likely to metastasize.

To identify a diagnostic marker which is improved over PSA, it is crucial to understand the biochemical differences between the malignant state and the benign state and between tumor cells with high and low metastatic potential. Overexpression and underexpression of certain cell adhesion molecules at the cell surface has been proposed to reflect metastasis of several cancers [Tang and Honn (1994-1995) *Invasion Metas.* 14, 109-122]. For example, the low expression of E-cadherin has been correlated with poor prognosis of prostate cancer [Rinker-Schaeffer et al. (1993) *supra*]. The present invention provides a positive correlation between the level of MUC18 expression and high metastatic potential of prostate cancer cells.

The human MUC18 (huMUC18) cDNA sequence (see Table 1A) obtained by the present inventors is different from the huMUC18 cDNA sequence given in GenBank Accession No. N28882 [Johnson et al. (1994)]. The deduced amino acid sequence of huMUC18 cDNA given in Table 1A was identical to the huMUC18 sequence deposited in GenBank by Johnson's group except seven amino acid residues. This discrepancy of amino acid sequence may be due to allelic differences. However, the amino acid sequence of the inventor's huMUC18 (646 amino acids) was very different from that published by Johnson's group in 1989 (603 amino acids), which 1989 sequence appears to contain sequence errors in the huMUC18 cDNA.

The human MUC18 cDNA sequence disclosed in Table 1A was amplified by RT-PCR from poly(A)+RNA, which was isolated from the human melanoma cell line SK-Mel-28. The cDNA was cloned into the pGEM-T-easy vector (Promega, Madison, WI). The DNA sequence of the huMUC18 cDNA was determined by rapid DNA sequencing using ABI prism dye terminator cycle sequencing ready reaction kit (Perkin-Elmer) with various

huMUC18 specific primers in an automated sequencer of ABI 373XL system. The Lasergen and GCG programs were used for comparison of nucleotide and amino acid sequences of the huMUC18 cDNA.

A cDNA sequence of a human MUC18 clone has been published in GenBank under
5 Accession No. M 28882 (See Table 1B). The translation initiation codon (ATG) is underlined, as is the translation termination codon (TAG). The PvuII (CAGCTG) and XhoI (CTCGAG) restriction sites are boxed, and cut sites are indicated by vertical arrows.

Table 1C (see also SEQ ID NO:5) shows the human MUC18 sequence as modified to
10 introduce a BamHI site (GGATCC) just upstream of the translation start site to facilitate cloning. The translation initiation (ATG) and termination (TAG) codons are boxed. The long arrows near the 5' and

TABLE 1A

HUMAN MUC18 cDNA SEQUENCE WITH DEDUCED AMINO ACID SEQUENCE

1	ATG GGG CTT CCC AGG CTG GTC TGC GGC TTC TTG CTC GGC TGC TGC TGT CCT CGC GTC GGG GGT GTG CCC GGA GAG GCT GAG CAG Met Gly Leu Pro Arg Leu Val Cys Ala Phe Leu Leu Ala Ala Cys Cys Pro Arg Val Ala Gly Val Pro Gly Glu Ala Glu Gln	90
91	CCT GCG CCT GAG CTG GAG GTG GAA GTG GGC AGC ACA GGC CTT CTG AAG TGC GGC CTC TCC CAG TCC CAA GGC AAC CTC AGC CAT GTC Pro Ala Pro Glu Leu Val Glu Val Glu Val Gly Ser Thr Ala Leu Leu Lys Cys Gly Leu Ser Gln Ser Gln Gly Asn Leu Ser His Val	180
181	GAC TGG TTT TCT GTC CAC AAG GAG AAG CGS ACG CTC ATC TTC COT GTG GGC CAG GGC CAG AGC GAA COT GGG GAG TAC GAG CAG Asp Trp Phe Ser Val His Lys Glu Lys Arg Thr Leu Ile Phe Arg Val Arg Gln Gly Gln Gly Ser Glu Pro Gly Tyr Glu Gln	270
271	CGC CTC AGC CTC CAG GAC AGA GGG GCT ACT CTG GGC CTG ACT CAA GTC ACC CCC CAA GAG CAG CAC ATC TTC TTG TGC CAG GGC AAG CGC Arg Leu Ser Leu Gln Asn Asp Arg Gly Ala Thr Leu Ala Leu Thr Gln Asn Asp Glu Arg Ile Phe Leu Cys Gln Gly Lys Arg	360
361	CCT CGG TCC CAG GAG TAC CGC ATC CAG CTC CGC GTC TAC AAA GCT CGC CAG CCA AAC ATC CAG GTC AAC CCC CTG GGC ATC CTT GTG Pro Arg Ser Gln Glu Tyr Arg Ile Gln Leu Arg Val Tyr Lys Ala Pro Glu Glu Pro Asn Ile Gln Val Asn Pro Leu Gly Ile Pro Val	450
451	AAC AGT AAG GAG CCT GAG GAG GTC GCT ACC TGT GTA GGG AAG AAC GGG TAC CCC ATT CCT CAA GTC ATC TGG TAC AAG AAT GGC CGG CCT Asn Ser Lys Glu Pro Glu Glu Val Ala Thr Cys Val Gly Arg Asn Gly Tyr Pro Ile Pro Gln Val Ile Trp Tyr Lys Asn Gly Arg Pro	540
541	CTG AAG GAG AAG AAC CGS GTC CAC ATT CAG TCG TCC CAG ACT GTG CAG TCG AGT GGT TTT TAC ACC TTG CAG AGT ATT CTG AAG GCA Leu Lys Glu Glu Lys Asn Arg Val His Ile Gln Ser Ser Gly Leu Tyr Thr Leu Gln Ser Ile Leu Lys Ala	630
631	CAG CTG GTT AAA GAA GAC AAA GAT GCC GAT TTT TAC TGT GAG CTC AAC TAC CGS CTG CCC AGT GGG AAC CAC ATG AAG GAG TCC AGG GAA Gln Leu Val Lys Glu Asp Lys Asp Ala Gln Phe Tyr Cys Glu Leu Asn Tyr Arg Leu Pro Ser Gly Asn His Met Lys Glu Ser Arg Glu	720
721	GTC ACC GTC CCT GTT TTC TAC CCG ACA GAA AAA GTG TGG CTG GAA GTG GGC CCG GTG GGA ATG CTG AAG GAA GGG GAC CGC GTG GAA ATC Val Thr Val Pro Val Phe Tyr Pro Thr Glu Lys Val Trp Leu Glu Val Glu Pro Val Gly Met Leu Lys Glu Gly Asp Val Glu Ile	810
811	AGG TGT TTG GCT GAT GGC AAC CCA CAC CAC TTC AGC ATC AGC AAC CCG AGC AAC CCG AGG GAG GCA GAG GAA ACA ACC AAC Arg Cys Leu Ala Asp Gly Asn Pro Pro Pro His Phe Ser Ile Ser Lys Gln Asn Pro Ser Thr Arg Glu Ala Glu Glu Thr Thr Asn	900
901	GAC AAC GGG GTC CTG GTG CTG GAG CCT GCC CGG AAG GAA CAC AGT GGG CCG TAT GAA TGT CAG GGC CTG GAC ACC ATG ATA TCG Asp Asn Gly Val Leu Val Leu Glu Pro Ala Arg Lys Glu His Ser Gly Arg Tyr Cys Gln Gly Leu Asp Thr Met Ile Ser	990
991	CTG CTG AGT GAA CCA CAG GAA CTA CTG GTG AAC TAT GTG TCT GAC GTC CGA GTG AGT CCC GCA GGC CCT GAG AGA CAG GAA GGC AGC AGC Leu Leu Ser Glu Pro Gln Glu Leu Leu Val Asn Tyr Val Ser Asp Val Arg Val Ser Pro Ala Ala Pro Glu Arg Gln Gly Ser Ser	1080
1081	CTC ACC CTG ACC TGT GAG GCA GAG AGT ACC CAG GAC CTC GAG TTC CAG TGG CTG AGA GAA GAG ACA GGC CAG GTG CTG GAA AGG GGG CCT Leu Thr Leu Thr Cys Glu Ala Glu Ser Ser Gln Asp Leu Glu Phe Gln Trp Leu Arg Glu Thr Gly Gln Val Leu Glu Arg Gly Pro	1170
1171	GTG CTT CAG TTG CAT GAC CTG AAA CGG GAG GCA GGA GGC GGC TAT CGC TGC GTG GCG TCT GTG CCC AGC ATA CCC GGC CTG AAC CGC ACA Val Leu Gln Leu His Asp Leu Lys Arg Glu Ala Gly Gly Tyr Arg Cys Val Ala Ser Val Pro Ser Ile Pro Gly Leu Asn Arg Thr	1260
1261	CAG CTG GTC AAC GTG GCC ATT TTT GGC CCC CCT TGG ATG GCA TTC AAG GAG AAG GTG TGG GTG AAA GAG AAT ATG GTG TTG AAT CTG Gln Leu Val Asn Val Ala Ile Phe Gly Pro Pro Trp Met Ala Phe Lys Glu Arg Lys Val Trp Val Lys Glu Asn Met Val Leu Asn Leu	1350
1351	TGT TGT GAA CGG TCA GGG CAC CCC CGG CCC ACC ATC TCC TGG AAC GGC ACG GCA AGT GAA CAA GAC CAA GAT CCA CAG CGA GTC Ser Cys Glu Ala Ser Gly His Pro Arg Pro Thr Ile Ser Trp Asn Val Asn Gly Thr Ala Ser Glu Glu Asp Pro Gln Arg Val	1440
1441	CTG AGC ACC CTG AAT GTC CTC GAG CTG ACC CGG GAG CTG TTG GAG ACA GGT GTT GAA TGC ACG GGC TCC AAC GAC CTG GGC AAA AAC ACC AGC Leu Ser Thr Leu Asn Val Leu Val Thr Pro Glu Leu Glu Thr Gly Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser	1530
1531	ATC CTC TTC CTG GAG CTG GTC AAT TTA ACC ACC CTC ACA CCA GAC TCC AAC ACA ACC ACT GGC CTC AGC ACT TCC ACT GGC CCT CAT Ile Leu Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser Asn Thr Thr Thr Gly Leu Ser Thr Thr Ala Ser Pro His	1620
1621	ACC AGA GCC AAC AGC ACC TCC ACA GAG AGA AAG CTG CGS GAG CGS GAG AGC CGG GGC GTG GTC ATC GTG CCT GTG TGC ATC CTG Thr Arg Ala Asn Ser Thr Thr Glu Arg Lys Leu Leu Pro Glu Pro Glu Ser Arg Gly Val Val Val Ala Val Ile Val Cys Ile Leu	1710
1711	GTC CTG GCG GTG CTG GGC GCT GTC CTC TAT TTC CTC TAT AAG AAG GGC AAG CTG CGS TCG AGC CGC TCA GGG AAG CAG GAG ATC AGC CTG Val Leu Ala Val Leu Gly Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys Arg Arg Ser Gly Lys Gln Glu Ile Thr Leu	1800
1801	CCC CGG TCT GAT AAG AGC GAA CTT GTA GTT GAA GTT AAG TCA GAT AAG CTC CCA GAA GAG ATG GGC CTC CTG CAG GGC AGC GGT GAC Pro Pro Ser Arg Lys Ser Glu Leu Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Met Gly Leu Leu Gln Gly Ser Ser Gly Asp	1890
1891	AAG AGG GCT CGG GGA GAC CAG GGA AAG AAA TAC ATC GAT CTG AGG CAT TAG CCCCCAAAT Lys Arg Ala Pro Gly Asp Gln Gly Lys Tyr Ile Asp Leu Arg His End	

HUMAN MUC18 cDNA (GENBANK ACCESSION NO. M28882).

TABLE 1B

GGGAAGCATGGGGCTCCAGGCTGGTCTGCGCCTTCTTGCTCGCCGCCCTGCTGCTGCTCCTCGCGTC
GCGGGTGTGCCCCGAGAGGCTGAGCAGCCTGCGCCTGAGCTGGTGGAGGTGGAAGTGGGAGCAGACAGCCC
TTCCTGAAGTGGGCCCTCTCCAGTCCCAAGGCAACCTCAGCCATGTCGACTGTTTCTGTCTCCACAAGGA
GAAGCGGACGCTCATCTTCCGTGTGCGCCAGGGCCAGGCCAGAGCGAACTGGGGAGTACGAGCAGCGG
CTCAGCCTCCAGGACAGAGGGCTACTCTGGCCCTGACTCAAGTCAACCCCTCCAGCTCCGCTCTACAAAGCTCCGGAGGA
TGTCAGGGCAAGCGCCCTCGGTCCAGGAGTACCGCATCCAGCTCCGCTGAGGAGCTGAGGAGTCCGTACCTGT
GCCAAACATCCAGGTCAACCCCTGGGCATCCCTGTGAACAGTAAGGAGCTGAGGAGTCCGTACCTGT
GTAGGGAGGAACGGGTACCCCATTCCTCAAGTCACTGTGGTACAAGAAATGGCCGCCCTCTGAAGGAGGAGA
AGAACC GGTCACATTCAGTCTGTCCTCAGACTGTGGAGTCGAGTGGTTTGTACACCTTGCAGAGTATTCT
GAAGGCACAGCTGTTAAAGAAGACAAAGATGCCAGTTTACTGTGAGCTCAACTACCGGCTGCCCAGT
GGAAACCATGAAGGATCCAGGGAAGTCAACGTCCTGTTTCTACCCGACAGAAATAAGTGTGGCTGG
AAGTGGAGCCCGTGGGAATGCTGAAGGAAGGGACCGCGTGGAAATCAGGTGTTTGGCTGATGGCAACCC
TCCACCAACACTTCAGCATCAGCAAGCAGAAACCCAGCACAGGAGGAGGAGAAAGAGACAACCAACGAC
AACGGGTCTGTGTCGTGGAGCTGCCCGAAGGAACACAGTGGCGCTATGAATGTACGCTCCGAACT
TGGACACCATGATATCGCTGCTGAGTGAACCAAGGAACCTACTGGTGAACCTATGTGTCTGACGTCGAGT
GAGTCCCGCAGCCCTGAGAGACAGGAAGGACAGCAGCCCTCACCTGACCTGTGAGGCAGAGAGTAGCCAG
GACCTCGAGTCCAGTGGCTGAGAGAAGAGACAGACAGCAGTGTGGAAAGGGGCCCTGTGCTTCAGTTGC
ATGACCTGAACGGGAGGACAGGAGCGGCTATCGCTGCGTGGCTGTGTGCCCAGCATACCCGGCCTGAA
CCGCACACAGCTGGTCAAGCTGGCCATTTTGGCCCCCTTGGATGGCATTCAGGAGAGGAAGGTGTGG
GTGAAAGAGAAATATGGTGTGAACTCTGTCTTGTGAAGCGTCAGGGCACCCCGGCCACCATCTCCTGGA
ACGTCAACGGCACGGCAAGTGAAACAAGACCAAGATCCACAGCGAGTCTTGAGCACCTGAATGTCTCCTCGT
GACCCCGGAGCTGTTGGAGACAGGTGTTGAATGACAGGCTCCACAGCTGGCAAAACACACAGCATC
CTCTTCTTGAGCTGGTCAATTTAACCAACCTCACCAGACTCCACACAAACCACTGGCCCTCAGCACTT
CCACTGCCAGTCTCATACAGAGCCAAACAGCACCTCCACAGAGAGAAAGCTGCCGGAGCCGGAGAGCCG
GGCGTGGTCATCGTGGCTGTGATTGTGTCATCTGTGTCCTGGCGGTGCTGGCGCTGTCTCTATTTC
CTCTATAAGAAGGCAAGTGCCTGTCAGGCGCTCAGGGAAGCAGGAGATCACGCTGCCCGCTCTCGTA
AGACCGAACTTGTAGTTGAAGTTAAGTCAGATAAGCTCCAGAAAGAGATGGGCCCTCCTGCAGGGCAGCAG
CGGTGACAAAGAGGGCTCCGGGAGACCAGGGAGAGAAATACATCGATCTGAGGCATTAGCCCGGAATCACT

3' ends indicate primer positions (BF1 and ER6a, respectively). The cut site for BamHI within its recognition sequence (GGATCC) is indicated with a vertical arrow.

The GenBank M 28882 sequence (given in SEQ ID NO:3) is identical to a human MUC18 cDNA clone (huMUC18) from human SK-Mel-28 cells, a human malignant melanoma cell line which produces relatively high levels of the MUC18 protein. This sequence is slightly different from the huMUC18 previously published [Johnson and Rummel (1996) in *Immunology of Human Melanoma*, ed, Maio, M., IOA Press, Washington, DC, pp. 31-38; Lehmann et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 9891-9895; Luca et al. (1993) *Melanoma Res.* 3, 35-41]. The two cDNAs have three stretches of amino acid (aa) residues that are different, such as 19 aa and 17 aa at the N-terminal portion and 17 aa near the C-terminal portion. Furthermore, the published human MUC18 cDNA sequence was missing 42 amino acids at the C-terminal end (see SEQ ID NO:4). The human and murine cDNAs have 74.5% identity in the deduced amino acid sequences. The 3'-end primer used previously, ER6, did not include the last few codons and the termination codon. To re-clone the intact correct human MUC18 cDNA, a correct new 3'-primer, ER6a, for amplifying the intact human MUC18 cDNA was designed (see hereinbelow).

Efforts to express the recombinant huMUC18 protein in the pCal-n expression system (Stratagene, La Jolla, CA) in *E. coli* failed. Finally, the expression was possible by using a GST-fusion protein expression system. The huMUC18 cDNA was cloned in the PGEX-6p-1 vector (Pharmacia), a small amount of the nearly intact MUC18 protein in *E. coli* was expressed. Fortunately, the sequence of the middle portion of the huMUC18 cDNA was correct, and it was then used for making recombinant protein in *E. coli*. Only when the middle 166 amino acid portion encoded by the cDNA, but not the N-terminal or C-terminal portions, was used for expression, over-expression of the recombinant protein was possible.

One pair of primers: BamHI-HMUC18-pvuII (28-mer, GGATCCCAGCTGGTTAAAGAAGACAAAG) (SEQ ID NO:6) and HMUC18-xhoI (27-mer, CTGGAACTCGAGGTCCTGGCTACTCTC) (SEQ ID NO:7) were used for PCR-amplification of the region from PvuII to XhoI of the HuMUC18 cDNA. The amplified fragment was cloned into pGEM-T Easy vector. The DNA fragment that included the coding region from the PvuII site to the XhoI site was excised from the pGEM-T Easy recombinant plasmid by two restriction enzymes, BamHI and Sall, and cloned into the BamHI and Sall

cleaved pGEX-6P-1 vector. The recombinant HuMUC18-middle fragment after cleavage with PrScission protease and purification contained the following sequences (see also Table 1A and SEQ ID NO:2).

	211	376
5	(Gly-Pro-Leu-Gly-Ser-)(SEQ ID NO:8) Gln-Leu- Leu-Glu-Phe-Gln- (Asn-His).	
	PGEX-6P-1 vector	(Amino acids 211-376 of SEQ ID NO:2) pGEM T Easy vector

A 22 kDa protein fragment was expressed from the PvuII to XhoI fragment of the cDNA (see Tables 1A and 1C). A large-scale preparation of the recombinant human MUC18 "middle portion" fragment was carried out. More than 6 mg of the purified recombinant protein was obtained after purification through a glutathione-affinity column, cleavage with the HRV-3C protease, being eluted and concentrated, and further purification through a Superdex column in a Pharmacia FPLC system. After final concentration of the eluant, 6 mg of the "middle portion" recombinant protein was sent to Lampire Biological Laboratories to make polyclonal antibodies in chickens. High antibody titers were reported. Eggs are collected and IgY (chicken antibody protein) is purified from these eggs. After the titers of these purified IgY preparations are determined, they are used for immunological testing.

Using the MUC18-specific antibody preparation from chickens after immunization with the purified human recombinant huMUC18-middle fragment protein, the present inventor has shown that these antibodies can react with the human MUC18 protein expressed in human prostate cancerous cell lines and prostatic cancerous tissues by Western blot analysis. The results showed that the human MUC18 protein was only expressed in three metastatic prostate cancer cell lines (Tsu-PR-1, DU145 and PC-3), but not in one non-metastatic cell line (LNCaP.FGC). These results are consistent with the Northern blot analysis of the expression of huMUC18 mRNA in these prostatic cancer cell lines, described herein. The human MUC18 protein was weakly expressed in normal prostatic epithelial cells and in normal prostate gland, but highly expressed in human cancerous prostatic tissues, as shown by Western blot analysis of the extracts prepared from these tissues. See Fig. 6 for the results of Western blot analysis.

Further immunohistochemical analysis revealed that huMUC18 is expressed in the membrane of the expected special cell types, such as metastatic melanoma tissues, endothelial

cells, and smooth muscle cells. This indicates that the antibodies are very specific for huMUC18 antigen and work well with the formaldehyde-fixed, paraffin-embedded tissue sections. As expected, the antibodies did not react with any antigens of the normal secretory epithelial cells in the acini of the prostate gland. Interestingly, they react with the secretory epithelial cells in the acini of the prostate cancer tissues. The expression of human MUC18 protein (or antigen) only in cancerous epithelial cells, but not in normal epithelial cells, supports the use of human MUC18 as a diagnostic marker for the metastatic potential of prostate cancers.

Table 2 summarizes the results for MUC18 expression in four prostate cancer cell lines in comparison with pertinent results published by other groups. Expression of MUC18 in these cell lines is consistent with their low or no expression of E-cadherin and α -catenin and their extent of invasiveness *in vitro* and metastasis in nude mice. See Fig. 6 for Western blot analysis.

The present work has correlated relatively high levels of MUC18 with the ability of prostate cancer cells to metastasize. High levels of MUC18 expression were observed in the three metastatic prostate cancer cell lines TSU-PR1, DU145 and PC-3. MUC18 expression was not detectable in the LNCAP prostate cell line, which is not metastatic. Nonmetastatic prostate cancer cells and normal prostate cells produce no or barely detectable expression of MUC18 either as protein or mRNA. Experiments in which the LNCAP cell line is genetically engineered to express MUC18 at high levels demonstrate that when cells gain the capacity to express MUC18 at high levels, those cells gain the ability to metastasize. Experiments in which the nonmetastatic prostate cancer cell line LNCaP.FGC is genetically engineered to express MUC18 at high levels demonstrate that when the cells gain the capacity to express MUC18 at high levels, the ability to metastasize is also gained. Thus, the relative level of MUC18 expression in prostate tumor tissue is correlated with the ability to metastasize, and measurement of MUC18 expression in prostate tumor biopsy tissue allows the medical practitioner to choose the most appropriate therapy for each prostate cancer patient, with high levels of MUC18 expression mandating an aggressive treatment strategy, likely including surgery, chemotherapy and/or radiation.

TABLE 2
RELATIVE LEVELS OF MUC18 EXPRESSION

	TSU-PR1	DU145	PC-3	LNCAP	Reference
E-cadherin expression	0	0.1	0.6	1.1	Morton et al. (1993) <i>Cancer Res.</i> 53,3585-3590
α -catenin expression	none	none	none	yes	Morton et al. (1993)
Total RNA (MUC18)	yes	yes	yes	none	this work
tumor growth in nude mice	yes (2000)	yes (500)	Yes (1400)	yes (2000)	Passaniti et al. (1992) <i>International J. Cancer</i> 51,318-324
Metastasis in nude mice	yes	yes	yes	none	Lalani et al. (1992) <i>Cancer Metastasis Rev.</i> 16,29-66
Invasiveness <i>in vitro</i>	yes (220)	yes (25)	yes (20)	none	Passaniti et al. (1992)

Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with MUC18, may be made by methods well known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York. Also, recombinant immunoglobulins may be produced by methods known in the art, including but not limited to, the methods described in U.S. Patent No. 4,816,567. Monoclonal antibodies with affinities of 10^8 M^{-1} , preferably 10^9 to 10^{10} M^{-1} or more, are preferred.

Antibodies (polyclonal or monoclonal) specific for MUC18 are useful, for example, as probes for screening DNA expression libraries or for detecting the presence (and relative amounts) of MUC18 in a test sample, for example, prostate tumor biopsy tissue or a tissue slice of a metastatic prostate cancer, or cells in culture which were derived from a primary prostate cancerous tumor or a metastatic prostate cancer tumor. Desirably, the results are

normalized to cell number or to total cellular protein. Frequently, the polypeptides and antibodies are labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include, but are not limited to, radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include, but are not limited to, Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al. (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis et al. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218, Part I; Wu (ed.) (1979) *Meth Enzymol.* 68; Wu et al. (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; and Setlow and Hollaender (1979) *Genetic Engineering: Principles and Methods*, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

All references cited in the present application are incorporated by reference herein to the extent that they are not inconsistent with the present disclosure.

The following examples are provided for illustrative purposes, and are not intended to limit the scope of the invention as claimed herein. Any variations in the exemplified articles and/or methods which occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLES

Example 1. Recombinant MUC18 Production and Antibody Production

The human MUC18 cDNA (1970 bp, RT-PCR amplified fragment) and three sub-fragments have been cloned in-phase in a GST-fusion protein expression system, pGEX-6p-1 (Pharmacia), which contains the glutathione-S-transferase (GST) coding region as an affinity-tag for the inserted foreign protein at its C-terminus. Fig.1 and Fig. 5 show the four possible fusions: the whole region, the N-terminal fragment, the middle fragment, and the C-terminal fragment.

Only the middle fragment of the human MUC18 protein can be induced by IPTG to express in a high amount in *E. coli* K-12 strain BL-21. Thus, only this protein is further purified for immunization. When culture A_{600} reaches 0.6 (2 to 3 hours after 1/100 inoculation of an overnight culture in L-broth with ampicillin), the expression of the recombinant middle fragment of MUC18 protein fused to GST in recombinant *E. coli* is induced by addition of 0.1 mM of IPTG to 3-liter cultures (1.5 liters per 4-liter baffled flask). Two hours after addition of IPTG at 37°C, cells are harvested by centrifugation at 3,000 rpm (2,323 x g) for 20 min in a horizontal HG-4L rotor in Sorvall RC-3 centrifuge. The cell pellet is suspended in 40 ml of ice-cold PBS (10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 2.7 mM KCl, and 140 mM NaCl, pH 7.3) and then lysed with a prechilled French pressure cell at 800 psi. The lysate is clarified by centrifugation for two to three times at 13,000 rpm (21,000 x g) for 30 min in SS-34 rotor in Sorvall RC-2 centrifuge. The protein concentration of the clear crude lysate adjusted to 10 mg/ml protein (about 60 ml) was used as the starting material for purification. The recombinant MUC18 proteins are purified from the clear crude lysate by batchwise adsorption to the Glutathione-Sepharose 4B affinity resin (about 20 ml of 50% slurry) by inversion on an inversion shaker at room temperature for 30 min. The GST portion of the fusion protein mediates the binding of the protein to the resin via the glutathione. After twice washing with 10 volumes (50 ml per 5 ml packed resin) of 1 X PBS and followed by twice washing with 1 X PreCission protease cleavage buffer (50 mM TrisHCl, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) to remove unbound proteins, the fusion protein on the resin is cleaved with 100 units of HRV-3C protease (PreCission protease, 2 units/ μl , from Pharmacia) by rocking on an inversion shaker for 16 hours at 4°C. The resin was spun down at 2,000 rpm for 10 min in a Sorvall RC-32 centrifuge. The supernatant and three washings

(10 ml 1 X PBS per 10 ml resin), which contain the recombinant MUC18 protein, are then combined and concentrated by centrifuging through a Centricon-30 (Millipore/Amicon). The purity of the protein is characterized by SDS-PAGE (8 to 10% polyacrylamide gel, slab gel). The 70kDa contaminated protein is removed by passing through a Superdex 200 HR 10/30 column in 1 X PBS (void volume about 7 ml for a 20 ml packed column), and the fractions containing the recombinant middle fragment MUC18 protein (22 kDa) (eluted at about 15.5 ml) were pooled. Fig. 2 shows the SDS-PAGE results of recombinant huMUC18 protein in the GST-fusion system.

Six mg of protein is sent to Lampire Biological Lab. (Pipersville, PA) for immunizing three chickens. The anti-MUC18 antibody titers are determined by ELISA assay using the purified recombinant MUC18 as the antigen. Eggs collected during the period of high serum antibody titers are used for purification of chicken immunoglobulin IgY.

To confirm the association between MUC18 expression and metastatic ability of prostate cancer cells, the human MUC18 coding sequence is introduced into a non-metastatic, non-expressing human prostate cancer cell line (LNCAP), and clones with different levels of expression of MUC18 are isolated.

The human MUC18 cDNA has been cloned into pCR3.1 Uni (Promega, Madison, WI), a mammalian expression vector in which high levels of gene expression are driven by the human CMV-IE promoter. The human MUC18 cDNA is also cloned into a murine amphitrophic retrovirus expression vector, e.g. pZipNeoSVX [Cepko et al. (1984) *Cell* 37, 1053-1062] or LXSXN [Miller and Rosman (1989) *BioTechniques* 7, 980-990], in which LTR drives gene expression.

These MUC18 recombinant vectors are used to transfect a human prostate cancer cell line which does not express MUC18, for example the LNCaP.FGC cell line [Umbas et al. (1992) *Cancer Res.* 52, 5104-5109; Iizumi et al (1987) *J. Urol.* 137: 1304-1306]. The vectors are introduced into the cultured cells by lipofection [Felgner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 7413-7417] or by electroporation [Potter, H. (1988) *Anal. Biochem.* 174:361-373]. G418-resistant clones are selected and purified [Yuo et al. (1992) *Interviol.* 34, 94-104] in view of the kanamycin resistance coding sequence expressed under the control of the SV40 promoter in each of the vectors. Relative expression levels of MUC18 expression in

different clones are determined by western blotting with polyclonal antibodies (described herein). Metastatic abilities are determined as described herein.

Example 2. Determination of Metastatic Ability of Prostate Cancer Cells

The degree of motility and the invasiveness of prostate cancer cells are quantitated using published methods [Tucker et al. (1994) *Eur. J. Cell Biol.* 58, 28-290; Repesh, L.A. (1989) *Invasion and Metastasis* 9, 192-208]. The Costar transwell chamber contains an inner well with a porous polycarbonate membrane, with 3 μm pore sizes in the bottom of the well. This is tightly fitted to the outer well.

To determine the motility of human prostate cancer cells, 0.5×10^5 cells are seeded in the top well. The cells remain on the top well because the poly carbonate membrane only allows medium to pass through freely. After seeding and attachment, the cells in the top well gradually migrate through the pores in the polycarbonate membrane to the bottom side of the membrane. Eventually some cells establish growth at the bottom side of the membrane. When the pore size of the membrane is about 3 μm , it somewhat slows the movement of the cells from the top side of the membrane to the bottom side. Motility of the cells is measured over the next several consecutive days. The rate of motility of a given cell line can be determined quantitatively by counting the cell number at the bottom of the membrane after trypsinization. Using this *in vitro* method, the motility rates of PC-3 and PC-huMUC18 human prostate cancer cells, with and without an over-expression of the human MUC18 protein, respectively, are determined and compared.

For the invasiveness of the prostate cancer cells, a similar kind of chamber is used, except before seeding the cells to the top well, the polycarbonate membrane is pre-coated with matrigel that contains protein components of the basal membranes of blood vessels. When the concentration of matrigel is correct, the membrane thus formed is thick enough to form a barrier to stop the cells from penetrating immediately, but is thin enough to allow cells to gradually invade through the membrane and migrate to the bottom of the membrane. Matrigel, which contains protein components of the base membrane of blood cell membrane, such as laminin, collagen type IV, entactin (nedogen), and heparin sulfate proteoglycan, is available commercially through Collaborative Research (Bedford, MA). Each filter in each 6.5 mm well is coated with 100 μl of a 1:20 dilution of commercial Matrigel in cold DMEM

(about 30 µg per filter). Using a similar method of counting the cells, which grow and attach to the bottom of the membrane, the rate of invasiveness of a given cell line can be quantitated [Repesh, L.A. (1989) *supra*]. To count the cells at the bottom of the membrane, the bottom of the membrane is treated with trypsin to detach the cells, and the cell number is counted directly using a Hemacytometer, or the cells on the membrane is stained with trypan blue and counted directly using a microscope. Alternatively, the cells are labeled with 0.6 µCi of ¹²⁵I-iododeoxyuridine (5 Ci/mg) for 18-24 h (about 95% of cells) and seeded to the top of the transwell chamber. After 72 h of invasion, the cells at the bottom and the cells on the top are trypsinized. The total input radioactivity is determined and compared to the radioactivities associated with cells from the top and bottom chambers. In this way the percentage of cells invading through the membrane can be accurately quantitated. Invasion rate can thus be determined [Repesh, L.A. (1989) *Invasion & Metast.* 2:192-208]. This type of in vitro test has been demonstrated to produce results in agreement with that of the in vivo animal tests [Repesh, L.A. (1989) *supra*]. Using this in vitro method, the invasion rates of LNCAP and LNCAP-huMUC18 transformed or transfected to express MUC18 human prostate cells, with and without over-expression of the human MUC18 protein, respectively, are determined and compared.

For comparison, the metastasis rates of these prostate cells are also tested in vivo in athymic nude mice [van Weerden et al. (1996) *Am. J. Pathol.* 149, 1055-1062]. The effect of the different expression levels of human MUC18 in prostate cancer cells on metastatic ability of different clones is determined. Similar pairs of cells as used for in vitro assay are also used in animal tests. The cells are implanted subcutaneously into nude mice. The size of tumors after different times are measured. The time and extent of the tumor cell metastases to bone or to other organs are investigated [van Weerden et al. (1996) *supra*].

The experiments set forth above confirm that the relative level of MUC18 expression in prostate cancer cells correlates positively with metastatic ability.

Example 3. Transcriptional Expression of MUC18

Total cell RNA is prepared from cell lines or from prostate tumor tissue with the method of one step acid-guanidinium-thiocyanate-phenol-chloroform extraction [Chomczynski and Sacchi (1987) *Anal. Biochem.* 162, 156-159]. We have shown that this

method consistently yields good quality RNA for RT-PCR. Poly(A)+RNA is prepared from total RNA by purifying through an oligo(dT)-cellulose column [Aviv and Leder (1972) *J. Molec. Biol.* 134, 743; Wu et al. (1985) *Int. J. Biochem.* 17, 355-363].

Northern hybridization technology is used to determine MUC18 mRNA levels expressed in the four prostate cancer cell lines [Ausubel et al. (1987) *Current Protocols in Molecular Biology*, Section 4]. Total RNA is extracted from cell preparations, electrophoretically separated in a formaldehyde-impregnated agarose gel, blotted and hybridized to a ³²P-labeled human MUC18 cDNA [Feinberg and Vogelstein (1983) *Anal. Biochem.* 132, 6-13]. Relative levels of MUC18 mRNA are detected by radioautography. The amount of RNA loaded and transferred is estimated by the intensities of the 28S and 16S bands in each lane. Alternately, G3PDH mRNA can be estimated using a G3PDH-specific probe or 28S and 18S probes after stripping the MUC18-specific probe.

Quantitative RT-PCR methodology [Innis et al. (1990) *PCR Protocols*, Academic Press; Quantitative RT-PCR (1993) *Methods and Applications Book #3*, Clontech, Palo Alto, CA]. Human Sk-Mel-28 cells are used as positive control for this method.

Because the quantity of mRNA from a small amount of tissue is small, quantitative RT-PCR is used for quantifying MUC18 mRNA expression in tissues [Innis et al. (1990) *supra*]. The isolation kit from Boehringer-Mannheim (Indianapolis, IN) using magnetic beads is suitable for obtaining a small amount of poly(A)+RNA from prostate tissue. The quality of mRNA isolated with this kit is also excellent for translation and RT-PCR. The quantitative RT-PCR method is first established from using mRNA of cultured cells, as described above, and then is used for quantifying the expression of MUC18 mRNA in different prostate cancer tissues.

The RT step is standard: a 20 µl RT reaction contains 1 µg of poly(A)+RNA from the human melanoma cell line, SK-Mel-28, as template, 0.5 µg of oligo(dT)₁₆ as primer (Promega), 2 µl of 10X AMV-RT buffer (Promega, Madison, WI), 2 units of AMV-reverse transcriptase, 5 mM MgCl₂, 1 mM of dNTP mix (Promega), 1 unit of RNase inhibitor (Promega), and 50 µg/ml of acetyl BSA. The reaction was carried out at 42 to 48°C for one hour, and heated at 99°C for 5 min.

A 20 µl PCR reaction contained 2 µl of RT reaction mixture (containing the first-strain cDNA), 2 µl each of the two primers (20 pm/µl), 0.01 mM dNTPs, 1 µg of acetyl BSA,

2 µl of 10X PCR buffer with 15 mM MgCl₂ (Promega), and 0.5 units of Taq DNA polymerase (Promega, 5 units/µl). PCR cycles are as follows:

Hot start at 94°C 5 min, 80°C 30 sec

29 cycles of 94°C 30 sec, 64-66°C 30 sec, and 72°C 2 min

1 cycle of 94°C 30 sec, 64-66°C 30 sec, and 72°C 60 min

The sequences of the primers for amplification of the human MUC18 cDNA from poly(A)+RNA of human melanoma cell line SK-Mel-28 are as follows:

BF1 27-mer 5'-CTCGGGATCCATGGGGCTTCCCAGGCT (SEQ ID NO:9)

ER6A 25-mer 5'-TCGGGGCTAATGCCTCAGATCGATG (SEQ ID NO:10)

Example 4. Immunofluorescence Assay for MUC18

Where the anti-human MUC18 antibodies are made in chicken, fluorescence-tagged anti-chicken IgG are used for immunofluorescent staining. Tissue culture cells, or normal or cancer prostate tissue samples are fixed, and first reacted with the anti-human MUC18 antibodies, washed, and then reacted with the fluorescence-tagged rabbit anti-chicken antibodies [Umbas et al. (1992) *Canc. Res.* 52, 5104-5109]. The presence of human MUC18 on the surface of these cells or tissues readily detected using UV-fluorescence microscopy.

Human melanoma cells, Sk-Mel-28 (ATCC HTB 72), which express MUC18 a high level of MUC18 are used as the positive control. Human melanoma cells, WM115 (ATCC CRL 1675) which express no MUC18, are used as the negative control. Three human prostate cancer cell lines, LNCap.FGC, PC-3, and DU145 are available from American Type Culture Collection, Rockville, MD, as ATCC CRL 1740, ATCC CRL 1435 and ATCC HTB 81, respectively. One other cell line, TSU-PR1, isolated by Dr. Iizumi in Japan [Iizumi et al. (1987) *J. Urol.* 137, *J. Urol.* 137:1304-1306] and provided by Dr. John T. Isaacs, John Hopkins University, Baltimore, MD] was tested. TSU-PR1 cells are more metastatic than the other three cell lines [Graff et al. (1995) *Cancer Res.* 55, 5195-5199].

All four cell lines are grown as monolayer cultures in a 37°C incubator with an atmosphere of 5% CO₂. TSU-PR1 and LNCap.FGC cells are grown in RPMI 1640 supplemented with 10% fetal bovine serum. PC-cells are grown in F12K medium with 10% fetal bovine serum. DU145 cells are grown in EMEM medium supplemented with pyruvate, extra non-essential amino acids and vitamins, and 10% fetal bovine serum.

Biopsy samples are taken, fixed and subjected to immunoassay using polyclonal antibody specific for human MUC18 described hereinabove, as described in Wood et al. (1994) *Cancer* 74:2533-2540.

I CLAIM:

1. A method for identifying metastatic potential of a prostate cancer cell, said method comprising the step of:

detecting expression of a MUC18 coding sequence in a prostate cancer cell for which an identification of metastatic potential is sought relative to expression of a MUC18 coding sequence in a normal prostate cell, wherein a higher level of expression of the MUC18 coding sequence is positively correlated with metastatic potential of a prostate cancer cell,

whereby metastatic potential of a prostate cancer cell is deemed high when the level of expression of a MUC18 coding sequence is higher in said prostate cancer cell than in a normal prostate cell.

2. The method of claim 1, wherein said prostate cancer cell is from a biopsy tissue sample from a patient for whom a prediction of metastasis of prostate cancer is sought.
3. The method of claim 1, wherein expression of MUC18 coding sequence is determined by immunoassay.
4. The method of claim 3, wherein expression of the MUC18 coding sequence is determined by immunoassay using antibody made in an experimental laboratory animal in response to a MUC 18 antigen.
5. The method of claim 4, wherein the MUC18 antigen is a middle portion of MUC18.
6. The method of claim 5, wherein said middle portion of MUC18 has an amino acid sequence as given in SEQ ID NO:2, amino acids 211-376.

7. The method of claim 1, wherein expression of a MUC18 coding sequence is determined by Northern hybridization.
8. The method of claim 7, wherein a probe used in Northern hybridization comprises at least 15 contiguous nucleotides of SEQ ID NO:1.
- 5 9. The method of claim 8, wherein a probe used in Northern hybridization comprises a nucleotide sequence as given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:10.
10. The method of claim 1, wherein said expression of a MUC18 coding sequence is determined by a reverse transcriptase-polymerase chain reaction.
- 10 11. The method of claim 10, wherein a primer used in the reverse-transcriptase polymerase chain reaction comprises a nucleotide sequence as given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9 or SEQ ID NO:10.
12. The method of claim 1, wherein said prostate cancer cell is a cell line cell.
13. An immunoassay kit for diagnosing metastatic potential of a prostate cancer cell, said kit comprising an antibody made in response to immunization with an antigen consisting essentially of middle portion MUC18.
14. A nucleic acid vector comprising a nucleotide sequence encoding a middle portion MUC18 protein, said middle portion MUC18 protein consisting essentially of an amino acid sequence as given in SEQ ID NO:2, amino acids 211-376.
- 20 15. The nucleic acid vector of claim 14, wherein said vector comprises a nucleotide sequence encoding a middle portion MUC18 protein as given in SEQ ID NO:1, nucleotides 631-1128.

16. A recombinant host cell comprising the nucleic acid vector of claim 14.
17. A nucleic acid vector comprising a nucleotide sequence encoding a MUC18 protein, said MUC18 protein being characterized by an amino acid sequence as given in SEQ ID NO:2.
- 5 18. The nucleic acid vector of claim 17, wherein said nucleotide sequence encoding a MUC18 protein is as given in SEQ ID NO:1, nucleotides 1-1938.
19. A recombinant host cell comprising the nucleic acid vector of claim 17.

ABSTRACT

The present disclosure provides methods for the diagnosis of metastatic prostate cancer and/or the prediction of the metastatic ability of prostate cancer in prostate biopsy tissue. Metastatic ability of prostate cancer is positively correlated with the level of transcriptional and translational expression of the MUC18 coding sequence in the neoplastic tissue. Methods for the determination of MUC18 protein synthesis include Western blots, ELISA, radioimmunoassay, immunofluorescence, and other immunoassays using MUC18-specific antibody and suitable detection means. Methods for measurement of transcriptional expression of the MUC18 coding sequence include Northern hybridizations and quantitative reverse transcriptase-polymerase chain reaction analyses. Absence of or very low MUC18 expression in the prostate tumor tissue is associated with nonmetastatic cancer, while relatively high levels of MUC18 expression are predictive of prostate cancer which is likely to metastasize or which has already metastasized. The present disclosure provides an improved diagnostic tool to aid the medical community in the choice of appropriate treatment regimens for prostate cancer patients.

FIG. 1

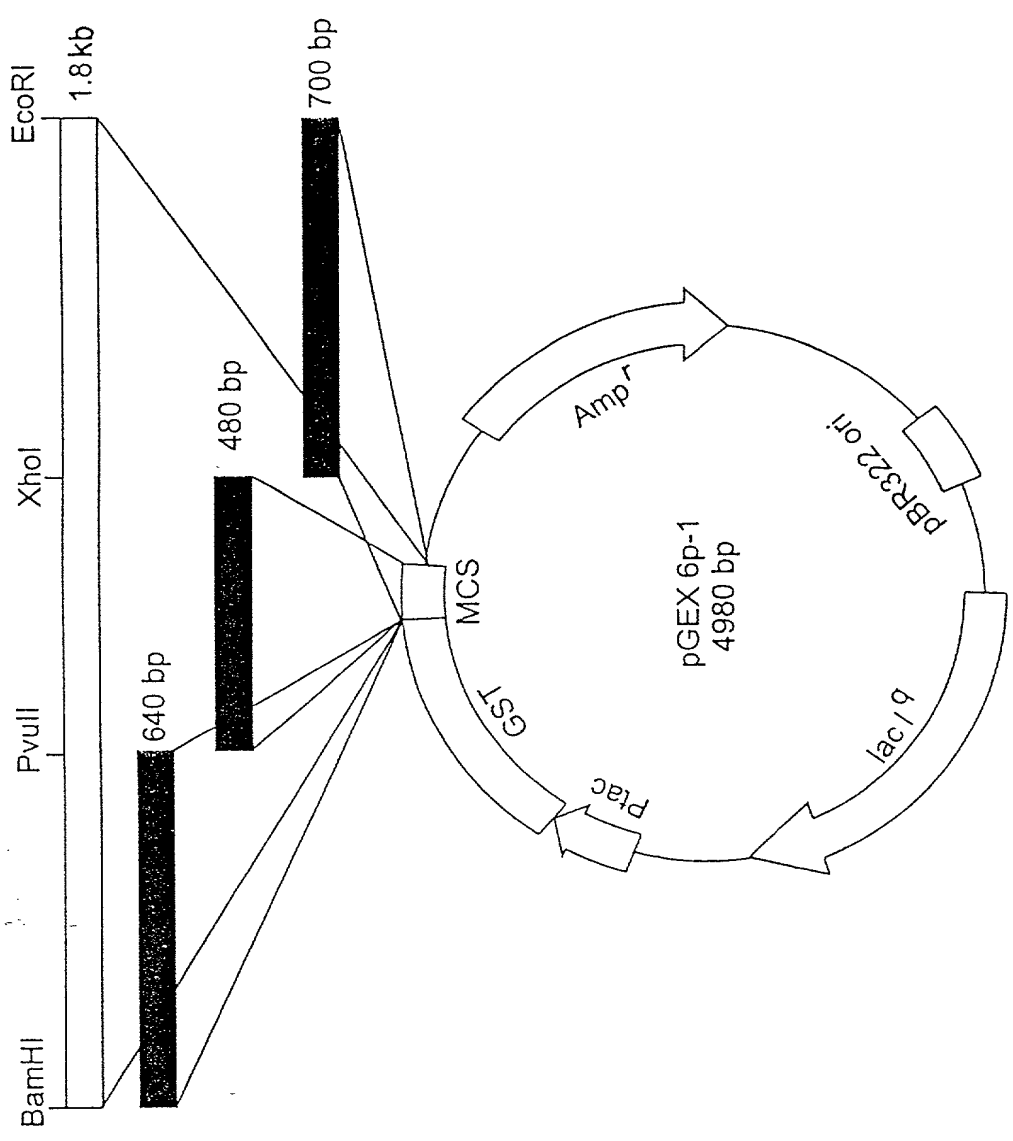
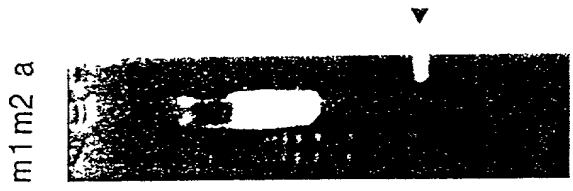
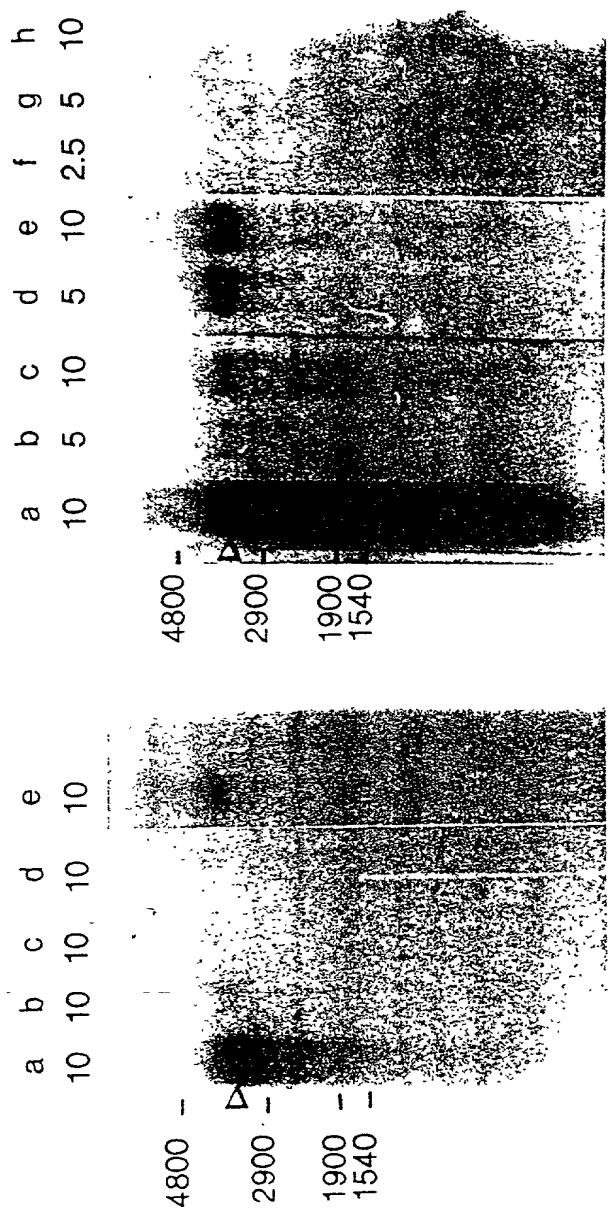


FIG. 1



2036-
1636-



SK, DU, TSUPRI, LNCAP

FIG. 2B

SK, DU, TSUPRI, LNCAP

FIG. 2A

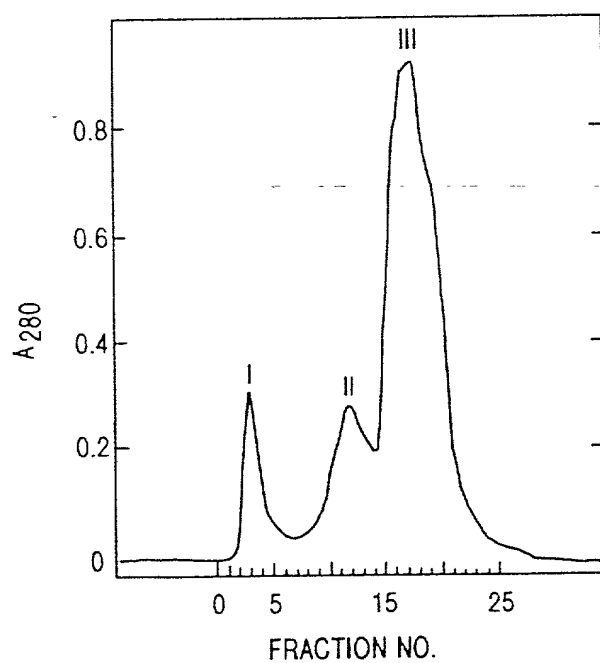
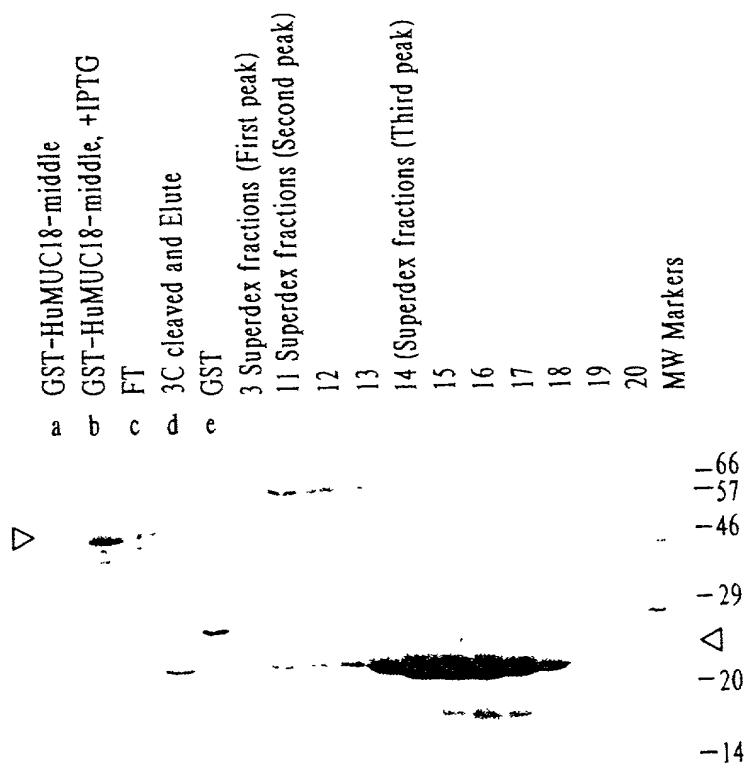


FIG. 3

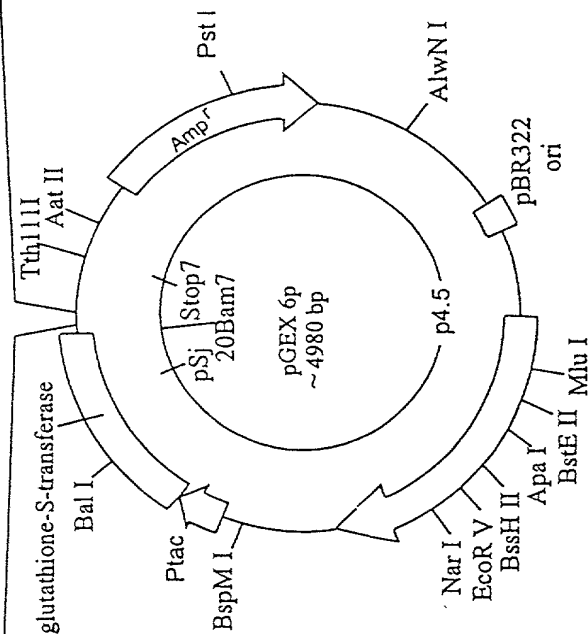
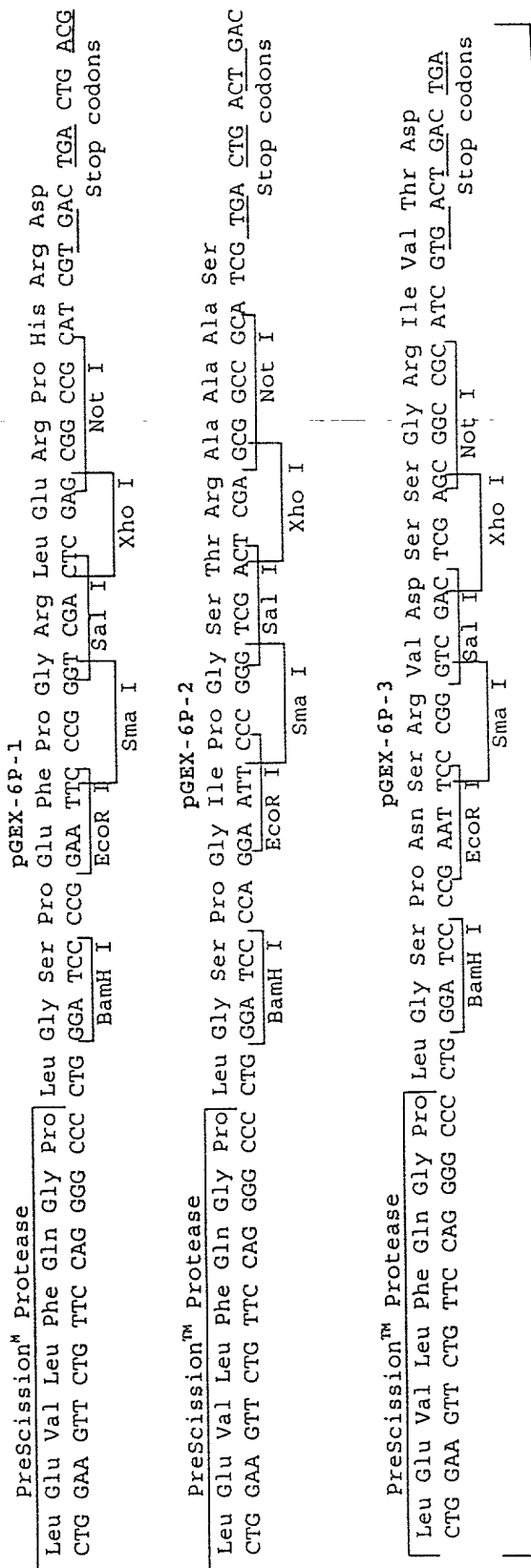


FIG. 4

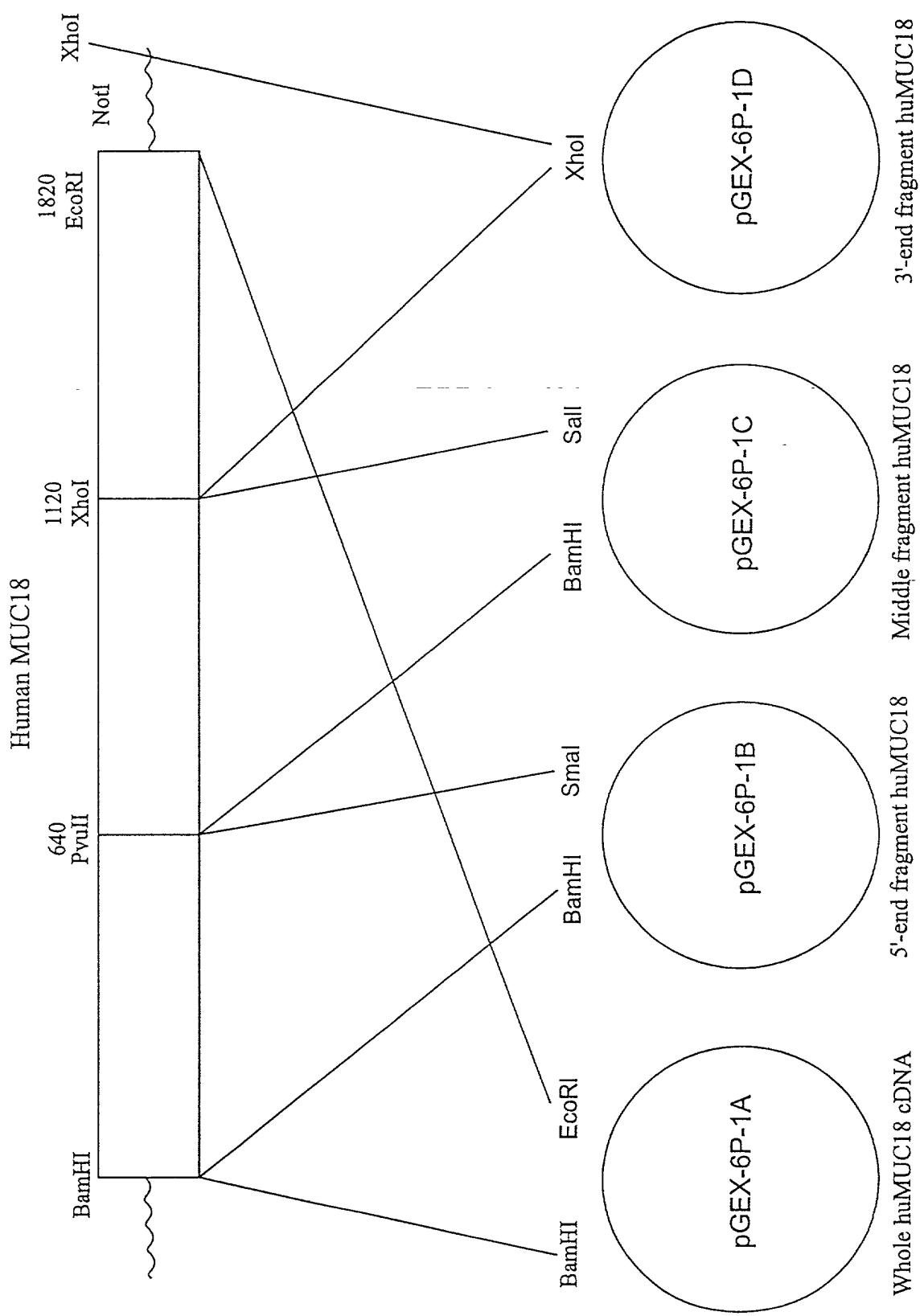


FIG. 5

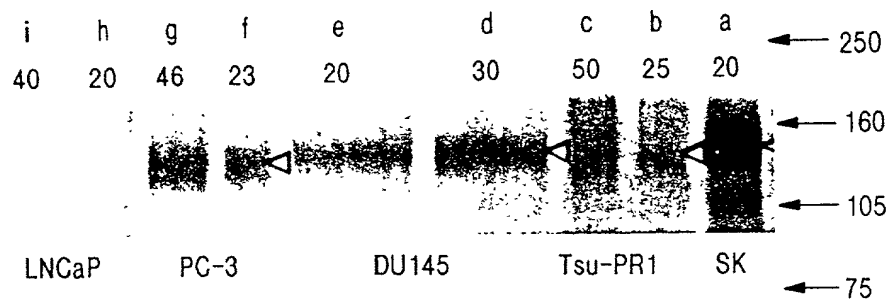


FIG. 6

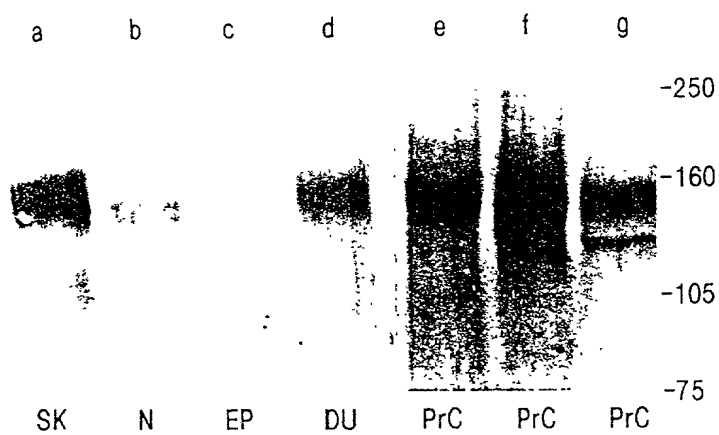


FIG. 7

INVENTOR'S DECLARATION FOR PATENT APPLICATION
AND POWER OF ATTORNEY

As the below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name;

I believe that I am the original, first and sole inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DIAGNOSTIC FOR METASTATIC PROSTATE CANCER,

the specification of which:

X is attached hereto.

_____ was filed on _____ as

and was amended on N/A (if applicable).

I hereby authorize our legal representative to add reference to the Serial No. and/or filing date of the above-referenced application to this declaration.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

Prior Foreign Application(s)

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

Country	Application No.	Date of Filing (day,month,year)	Date of Issue (day,month,year)	Priority Claimed 35 U.S.C.119
NONE				Yes__ No__

Prior Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application Serial Number	Date of Filing
60/076,664	3 March 1998

Prior U.S. Application(s) and PCT International Application(s) Designating the United States

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT International application(s) designating the United States listed below:

Application Serial Number	Date of Filing	Status
PCT US99/04850	2 March 1999	Pending

Insofar as the subject matter of each of the claims in this application is not disclosed in the prior United States, foreign or PCT International application(s) to which priority has been claimed above in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

I hereby appoint, both jointly and severally, as our attorneys and agents with full power of substitution and revocation, to prosecute this application and any corresponding application filed in the Patent Cooperation Treaty Receiving Office, and to transact all business in the Patent and Trademark Office connected herewith the following attorneys and agents, their registration numbers being listed after their names:

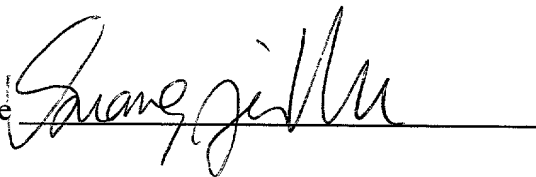
Lorance L. Greenlee, Reg. No. 27,894; Ellen P. Winner, Reg. No. 28,547; Sally A. Sullivan, Reg. No. 32,064; Donna M. Ferber, Reg. No. 33,878; G. William Van Cleave, Reg. No. 40,213; Susan K. Doughty, Reg. No. 43,595; and Heeja Yoo-Warren, Reg. No. 45,495, all of Greenlee, Winner and Sullivan, P.C., 5370 Manhattan Circle, Suite 201, Boulder, CO 80303.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of

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Residence: **3157 McCully Drive N.E., Atlanta, GA 30345**
Citizenship: **United States of America**
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Signature



Date

8/31/00

SEQUENCE LISTING

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Thr Gly Val Glu Cys Thr Ala Ser Asn Asp Leu Gly Lys Asn Thr Ser	
495 500 505 510	

atc ctc ttc ctg gag ctg gtc aat tta acc acc ctc aca cca gac tcc	1585
Ile Leu Phe Leu Glu Leu Val Asn Leu Thr Thr Leu Thr Pro Asp Ser	
515 520 525	
aac aca acc act ggc ctc agc act tcc act gcc agt cct cat acc aga	1633
Asn Thr Thr Thr Gly Leu Ser Thr Ser Thr Ala Ser Pro His Thr Arg	
530 535 540	
gcc aac agc acc tcc aca gag aga aag ctg ccg gag ccg gag agc cgg	1681
Ala Asn Ser Thr Ser Thr Glu Arg Lys Leu Pro Glu Pro Glu Ser Arg	
545 550 555	
ggc gtg gtc atc gtg gct gtg att gtg tgc atc ctg gtc ctg gcg gtg	1729
Gly Val Val Ile Val Ala Val Ile Val Cys Ile Leu Val Leu Ala Val	
560 565 570	
ctg ggc gct gtc ctc tat ttc ctc tat aag aag ggc aag ctg ccg tgc	1777
Leu Gly Ala Val Leu Tyr Phe Leu Tyr Lys Lys Gly Lys Leu Pro Cys	
575 580 585 590	
agg cgc tca ggg aag cag gag atc acg ctg ccc ccg tct cgt aag acc	1825
Arg Arg Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Thr	
595 600 605	
gaa ctt gta gtt gaa gtt aag tca gat aag ctc cca gaa gag atg ggc	1873
Glu Leu Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly	
610 615 620	
ctc ctg cag ggc agc agc ggt gac aag agg gct ccg gga gac cag gga	1921
Leu Leu Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly	
625 630 635	
gag aaa tac atc gat ctg agg cat tagccccgaa tcaact	1960
Glu Lys Tyr Ile Asp Leu Arg His	
640 645	

<210> 4

<211> 646

<212> PRT

<213> Homo sapiens

<400> 4

Met Gly Leu Pro Arg Leu Val Cys Ala Phe Leu Leu Ala Ala Cys Cys
1 5 10 15

Cys Cys Pro Arg Val Ala Gly Val Pro Gly Glu Ala Glu Gln Pro Ala
20 25 30

Pro Glu Leu Val Glu Val Glu Val Gly Ser Thr Ala Leu Leu Lys Cys
35 40 45

Gly	Leu	Ser	Gln	Ser	Gln	Gly	Asn	Leu	Ser	His	Val	Asp	Trp	Phe	Ser	
50						55					60					
Val	His	Lys	Glu	Lys	Arg	Thr	Leu	Ile	Phe	Arg	Val	Arg	Gln	Gly	Gln	
65					70					75					80	
Gly	Gln	Ser	Glu	Pro	Gly	Glu	Tyr	Glu	Gln	Arg	Leu	Ser	Leu	Gln	Asp	
				85					90					95		
Arg	Gly	Ala	Thr	Leu	Ala	Leu	Thr	Gln	Val	Thr	Pro	Gln	Asp	Glu	Arg	
		100						105					110			
Ile	Phe	Leu	Cys	Gln	Gly	Lys	Arg	Pro	Arg	Ser	Gln	Glu	Tyr	Arg	Ile	
	115						120					125				
Gln	Leu	Arg	Val	Tyr	Lys	Ala	Pro	Glu	Glu	Pro	Asn	Ile	Gln	Val	Asn	
130						135					140					
Pro	Leu	Gly	Ile	Pro	Val	Asn	Ser	Lys	Glu	Pro	Glu	Glu	Val	Ala	Thr	
145					150					155					160	
Cys	Val	Gly	Arg	Asn	Gly	Tyr	Pro	Ile	Pro	Gln	Val	Ile	Trp	Tyr	Lys	
				165					170					175		
Asn	Gly	Arg	Pro	Leu	Lys	Glu	Glu	Lys	Asn	Arg	Val	His	Ile	Gln	Ser	
			180					185					190			
Ser	Gln	Thr	Val	Glu	Ser	Ser	Gly	Leu	Tyr	Thr	Leu	Gln	Ser	Ile	Leu	
		195					200					205				
Lys	Ala	Gln	Leu	Val	Lys	Glu	Asp	Lys	Asp	Ala	Gln	Phe	Tyr	Cys	Glu	
210						215					220					
Leu	Asn	Tyr	Arg	Leu	Pro	Ser	Gly	Asn	His	Met	Lys	Glu	Ser	Arg	Glu	
225					230					235					240	
Val	Thr	Val	Pro	Val	Phe	Tyr	Pro	Thr	Glu	Lys	Val	Trp	Leu	Glu	Val	
				245					250					255		
Glu	Pro	Val	Gly	Met	Leu	Lys	Glu	Gly	Asp	Arg	Val	Glu	Ile	Arg	Cys	
			260					265					270			
Leu	Ala	Asp	Gly	Asn	Pro	Pro	Pro	His	Phe	Ser	Ile	Ser	Lys	Gln	Asn	
	275						280					285				
Pro	Ser	Thr	Arg	Glu	Ala	Glu	Glu	Glu	Thr	Thr	Asn	Asp	Asn	Gly	Val	
	290					295					300					
Leu	Val	Leu	Glu	Pro	Ala	Arg	Lys	Glu	His	Ser	Gly	Arg	Tyr	Glu	Cys	
305					310					315					320	

Gln	Ala	Trp	Asn	Leu	Asp	Thr	Met	Ile	Ser	Leu	Leu	Ser	Glu	Pro	Gln	
				325					330					335		
Glu	Leu	Leu	Val	Asn	Tyr	Val	Ser	Asp	Val	Arg	Val	Ser	Pro	Ala	Ala	
			340					345					350			
Pro	Glu	Arg	Gln	Glu	Gly	Ser	Ser	Leu	Thr	Leu	Thr	Cys	Glu	Ala	Glu	
		355					360					365				
Ser	Ser	Gln	Asp	Leu	Glu	Phe	Gln	Trp	Leu	Arg	Glu	Glu	Thr	Asp	Gln	
	370					375					380					
Val	Leu	Glu	Arg	Gly	Pro	Val	Leu	Gln	Leu	His	Asp	Leu	Lys	Arg	Glu	
385					390				395						400	
Ala	Gly	Gly	Gly	Tyr	Arg	Cys	Val	Ala	Ser	Val	Pro	Ser	Ile	Pro	Gly	
				405				410						415		
Leu	Asn	Arg	Thr	Gln	Leu	Val	Lys	Leu	Ala	Ile	Phe	Gly	Pro	Pro	Trp	
			420					425					430			
Met	Ala	Phe	Lys	Glu	Arg	Lys	Val	Trp	Val	Lys	Glu	Asn	Met	Val	Leu	
		435					440					445				
Asn	Leu	Ser	Cys	Glu	Ala	Ser	Gly	His	Pro	Arg	Pro	Thr	Ile	Ser	Trp	
	450						455				460					
Asn	Val	Asn	Gly	Thr	Ala	Ser	Glu	Gln	Asp	Gln	Asp	Pro	Gln	Arg	Val	
465					470					475					480	
Leu	Ser	Thr	Leu	Asn	Val	Leu	Val	Thr	Pro	Glu	Leu	Leu	Glu	Thr	Gly	
				485					490					495		
Val	Glu	Cys	Thr	Ala	Ser	Asn	Asp	Leu	Gly	Lys	Asn	Thr	Ser	Ile	Leu	
			500					505					510			
Phe	Leu	Glu	Leu	Val	Asn	Leu	Thr	Thr	Leu	Thr	Pro	Asp	Ser	Asn	Thr	
		515					520					525				
Thr	Thr	Gly	Leu	Ser	Thr	Ser	Thr	Ala	Ser	Pro	His	Thr	Arg	Ala	Asn	
	530					535					540					
Ser	Thr	Ser	Thr	Glu	Arg	Lys	Leu	Pro	Glu	Pro	Glu	Ser	Arg	Gly	Val	
545					550					555					560	
Val	Ile	Val	Ala	Val	Ile	Val	Cys	Ile	Leu	Val	Leu	Ala	Val	Leu	Gly	
				565					570					575		
Ala	Val	Leu	Tyr	Phe	Leu	Tyr	Lys	Lys	Gly	Lys	Leu	Pro	Cys	Arg	Arg	
			580					585					590			

Ser Gly Lys Gln Glu Ile Thr Leu Pro Pro Ser Arg Lys Thr Glu Leu
595 600 605

Val Val Glu Val Lys Ser Asp Lys Leu Pro Glu Glu Met Gly Leu Leu
610 615 620

Gln Gly Ser Ser Gly Asp Lys Arg Ala Pro Gly Asp Gln Gly Glu Lys
625 630 635 640

Tyr Ile Asp Leu Arg His
645

<210> 5
<211> 1962
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:HUMAN MUC18
cDNA, AS MODIFIED TO FACILITATE CLONING

<400> 5
ctcggatcca tggggcttcc caggctggtc tgcgccttct tgctcgccgc ctgctgctgc 60
tgtcctcgcg tcgcggtgt gcccgagag gctgagcagc ctgcgcctga gctggtggag 120
gtggaagtgg gcagcacagc ctttctgaag tgcggcctct ccagtccca aggcaacctc 180
agccatgtcg actggttttc tgtccacaag gagaagcgga cgctcatctt ccgtgtgcgc 240
cagggccagg gccagagcga acctggggag tacgagcagc ggctcagcct ccaggacaga 300
ggggctactc tggccctgac tcaagtcacc cccaagacg agcgcatctt cttgtgccag 360
ggcaagcgcc ctcggtccca ggagtaccgc atccagctcc gcgtctacaa agctccggag 420
gagccaaaca tccaggtcaa cccctgggc atccctgtga acagtaagga gcctgaggag 480
gtcgctacct gtgtagggag gaacgggtac cccattcctc aagtcacctg gtacaagaat 540
ggcgggcctc tgaaggagga gaagaaccgg gtccacattc agtcgtccca gactgtggag 600
tcgagtgggt tgtacacctt gcagagtatt ctgaaggcac agctgggtta agaagacaaa 660
gatgccaggt ttactgtga gctcaactac cggctgccca gtgggaacca catgaaggag 720
tcagggaag tcaccgtccc tgttttctac ccgacagaaa aagtgtggct ggaagtggag 780
cccggtggaa tgctgaagga aggggaccgc gtggaaatca ggtgtttggc tgatggcaac 840
cctccaccac acttcagcat cagcaagcag aaccccagca ccagggaggc agaggaagag 900

acaaccaacg acaacggggt cctggtgctg gagcctgccc ggaaggaaca cagtgggctgc 960
 tatgaatgtc aggcctggaa cttggacacc atgatatcgc tgctgagtga accacaggaa 1020
 ctactggtga actatgtgtc tgacgtccga gtgagtcccg cagcccctga gagacaggaa 1080
 ggcagcagcc tcaccctgac ctgtgaggca gagagtagcc aggacctcga gttccagtgg 1140
 ctgagagaag agacagacca ggtgctggaa agggggcctg tgcttcagtt gcatgacctg 1200
 aaacgggagg caggaggcgg ctatcgctgc gtggcgctctg tgcccagcat acccggcctg 1260
 aaccgcacac agctggtcaa gctggccatt tttggcccc cttggatggc attcaaggag 1320
 aggaaggtgt ggggtgaaaga gaatatggtg ttgaatctgt cttgtgaagc gtcagggcac 1380
 ccccggtcca ccattctctg gaacgtcaac ggcacggcaa gtgaacaaga ccaagatcca 1440
 cagcgagtcc tgagcaccct gaatgtcttc gtgaccccg agctgttga gacaggtgtt 1500
 gaatgcacgg cctccaacga cctgggcaaa aacaccagca tcctcttcct ggagctggtc 1560
 aatttaacca cctcacacc agactccaac acaaccactg gcctcagcac ttccactgcc 1620
 agtctcata ccagagccaa cagcacctcc acagagagaa agctgccgga gccggagagc 1680
 cggggcgctg tcacgtggc tgtgattgtg tgcattctgg tcctggcggt gctgggcgct 1740
 gtcctctatt tcctctataa gaagggaag ctgccgtgca ggcgtcagg gaagcaggag 1800
 atcacgtgc ccccgctctg taagaccgaa cttgtagttg aagttaagtc agataagctc 1860
 ccagaagaga tgggcctcct gcagggcagc agcggtgaca agagggtcc gggagaccag 1920
 ggagagaaat acatcgatct gaaggcatta gccccgaatc at 1962

<210> 6

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial

Sequence: OLIGONUCLEOTIDE, FOR SUBCLONING HUMAN
MUC18 FRAGMENT

<400> 6

ggatcccagc tggttaaaga agacaaag

28

<210> 7

<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial
Sequence:OLIGONUCLEOTIDE, FOR SUBCLONING HUMAN
MUC18

<400> 7
ctggaactcg aggtcctggc tactctc

27

<210> 8
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:AMINO ACID
SEQUENCE ENCODED BY PORTION OF PGEX-6P-1 VECTOR

<400> 8
Gly Pro Leu Gly Ser
1 5

<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial
Sequence:OLIGONUCLEOTIDE, SEQUENCE CORRESPONDS TO
HUMAN MUC18

<400> 9
ctcgggatcc atggggcttc ccaggct

27

<210> 10
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial
Sequence:OLIGONUCLEOTIDE, SEQUENCE CORRESPONDS TO
HUMAN MUC18

<400> 10

tcgggggctaa tgcctcagat cgatg

25

<210> 11

<211> 81

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:JUNCTION
SEQUENCE FOR MCU18 CLONED INSERT

<400> 11

ctggaagtgc tgttccaggg gccctggga tccccggaat tccccgggtcg actcgagcgg 60

ccgcacgtg actgactgac g

81

<210> 12

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:JUNCTION
SEQUENCE FOR CLONED MUC18 INSERT

<400> 12

Leu	Glu	Val	Leu	Phe	Gln	Gly	Pro	Leu	Gly	Ser	Pro	Glu	Phe	Pro	Gly
1				5				10					15		

Arg	Leu	Glu	Arg	Pro	His	Arg	Asp
			20				

<210> 13

<211> 81

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:JUNCTION
SEQUENCE FOR CLONED MUC18 INSERT

<400> 13

ctggaagtgc tgttccaggg gccctggga tccccaggaa tccccgggtc gactcgagcg 60

gccgcacgtg gactgactga c

81

<210> 14

<211> 23

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:JUNCTION
SEQUENCE IN FUSION PROTEIN

<400> 14
Leu Glu Val Leu Phe Gln Gly Pro Leu Gly Ser Pro Gly Ile Pro Gly
1 5 10 15
Ser Thr Arg Ala Ala Ala Ser
20

<210> 15
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:JUNCTION
SEQUENCE IN VECTOR WITH MCU18 CLONED INSERT

<400> 15
ctggaagttc tgttccaggg gccctggga tccccgaatt cccgggtcga ctcgagcggc 60
cgcatcgtga ctgactga 78

<210> 16
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:JUNCTION OF
FUSION PEPTIDE

<400> 16
Leu Gly Val Leu Phe Gln Gly Pro Leu Gly Ser Pro Asn Ser Arg Val
1 5 10 15
Asp Ser Ser Gly Arg Ile Val Thr Asp
20 25